Claims

What is claimed is:

1. A process for preparing a seeded dermal equivalent comprising:
   a. providing a dermal equivalent, keratinocytes, and culture media having an air interface;
   b. culturing said dermal equivalent in said culture media;
   c. lifting said dermal equivalent to said air interface to provide a lifted dermal equivalent; and
   d. seeding said dermal equivalent with said keratinocytes to provide a seeded dermal equivalent.

2. The process of Claim 1, wherein said keratinocytes are selected from the group consisting of primary human keratinocytes and immortalized human keratinocytes.

3. The process of Claim 2, wherein said immortalized keratinocytes are NIKS cells.

4. The process of Claim 1, wherein said dermal equivalent comprises collagen and fibroblasts.

5. The process of Claim 4, wherein said collagen is rat tail tendon collagen.

6. The process of Claim 4, wherein said fibroblasts are NHDF cells.

7. The process of Claim 1, wherein said lifting further comprises incubating said lifted dermal equivalent at said air interface for at least six hours prior to said seeding.

8. The process of Claim 1, wherein said lifting further comprises incubating said lifted dermal equivalent at said air interface for at least twelve hours prior to said seeding.

9. The process of Claim 1, wherein said lifting further comprises incubating said lifted dermal equivalent at said air interface for at least eighteen hours prior to said seeding.

10. The process of Claim 1, wherein said lifting further comprises incubating said lifted dermal equivalent at said air interface for about 24 hours prior to said seeding.

11. The process of Claim 1, wherein said lifting further comprises incubating said lifted dermal equivalent at said air interface for about six hours to about 24 hours prior to said seeding.

12. The process of Claim 1, further comprising step e) incubating said seeded dermal skin equivalent under conditions such that a skin equivalent is formed.

13. The process of Claim 12, wherein said skin equivalent is stratified.

14. The process of Claim 12, wherein said skin equivalent is stratified into squamous epithelia.

15. The seeded dermal equivalent produced by the process of Claim 1.

16. The skin equivalent produced by the process of Claim 12.

17. A process for preparing a skin equivalent comprising:

a. providing a dermal equivalent, keratinocytes, and culture media having an air interface;

b. culturing said dermal equivalent in said culture media;

c. lifting said dermal equivalent to said air interface to provide a lifted dermal equivalent;

d. seeding said dermal equivalent with said keratinocytes to provide a seeded dermal equivalent;

e. and culturing said seeded dermal equivalent under conditions such that a skin equivalent is formed.

18. The process of Claim 17, wherein said keratinocytes are selected from the group consisting of primary keratinocytes and immortalized keratinocytes.

19. The process of Claim 18, wherein said immortalized keratinocytes are NIKS cells.

20. The process of Claim 17, wherein said dermal equivalent comprises collagen and fibroblasts.

21. The process of Claim 20, wherein said collagen is rat tail tendon collagen.

22. The process of Claim 20, wherein said fibroblasts are NHDF cells.

23. The process of Claim 17, wherein said lifting further comprises incubating said lifted dermal equivalent at said air interface for at least six hours prior to said seeding.

24. The process of Claim 17, wherein said lifting further comprises incubating said lifted dermal equivalent at said air interface for at least twelve hours prior to said seeding.

25. The process of Claim 17, wherein said lifting further comprises incubating said lifted dermal equivalent at said air interface for at least eighteen hours prior to said seeding.

26. The process of Claim 17, wherein said lifting further comprises incubating said lifted dermal equivalent at said air interface for about 24 hours prior to said seeding.

27. The process of Claim 17, wherein said lifting further comprises incubating said lifted dermal equivalent at said air interface for about six hours to about 24 hours prior to said seeding.

28. The process of Claim 17, wherein said skin equivalent is stratified.

29. The process of Claim 17, wherein said skin equivalent is stratified into squamous epithelia.

30. A composition comprising a growth chamber having a porous bottom surface and a side-wall, said porous bottom having thereon a skin equivalent wherein said dermal equivalent is substantially adhered to said side-wall of said growth chamber.

31. The composition of Claim 30, wherein said porous bottom of said growth chamber is about 0.1 to 20.0 centimeters in diameter.

32. The composition of Claim 30, wherein said porous bottom of said growth chamber is about 1.0 centimeters in diameter.

33. The composition of Claim 30, wherein said skin equivalent is stratified.

34. The composition of Claim 30, wherein said skin equivalent stratified into squamous epithelia.

35. The composition of Claim 30, wherein said skin equivalent comprises a dermal equivalent.

36. The composition of Claim 35, wherein said dermal equivalent comprises collagen and fibroblasts.

37. The composition of Claim 36, wherein said collagen is rat tail tendon collagen.

38. The composition of Claim 36, wherein said fibroblasts are NHDF cells.

39. The composition of Claim 30, wherein said skin equivalent comprises keratinocytes.

40. The composition of Claim 39, wherein said keratinocytes are selected from the group consisting of primary keratinocytes and immortalized keratinocytes.

41. The composition of Claim 40, wherein said keratinocytes are NIKS cells.

42. A composition comprising a dermal equivalent, wherein said dermal equivalent contains about 0.2 mg collagen per square centimeter of said dermal equivalent to 2.0 mg collagen per square centimeter of said dermal equivalent.

43. The composition of Claim 42, wherein said dermal equivalent contains about 0.22 mg collagen per square centimeter of said dermal equivalent to 1.0 mg collagen per square centimeter of said dermal equivalent.

44. The composition of Claim 42, wherein said dermal equivalent contains about 0.5 mg collagen per square centimeter of said dermal equivalent.

45. The composition of Claim 42, further comprising keratinocytes.

46. The composition of Claim 45, wherein said keratinocytes are selected from the group consisting of primary keratinocytes and immortalized keratinocytes.

47. The composition of Claim 46, wherein said keratinocytes are NIKS cells.

48. The composition of Claim 42, wherein said dermal equivalent comprises collagen and fibroblasts.

49. The composition of Claim 48, wherein said collagen is rat tail tendon collagen.

50. A composition comprising BSA, isoproterenol, carnitine, serine, oleic acid, linoleic acid, arachidonic acid, α-tocopherol, ascorbic acid, and EGF.

51. The composition of Claim 50, wherein said BSA, isoproterenol, carnitine, serine, oleic acid, linoleic acid, arachidonic acid, α-tocopherol, ascorbic acid, and EGF are present in concentrations sufficient to improve barrier function in *in vitro* cultured skin equivalents.

52. The composition of Claim 50, further comprising serum.

53. The composition of Claim 50, further comprising calcium chloride, hydrocortisone, cholera toxin, insulin and adenine.

54. The composition of Claim 50, wherein said bovine serum albumin is provided at a concentration of about 0.1 – 10.0 mg/ml.

55. The composition of Claim 50, wherein said isoproterenol is provided at a concentration of about 0.1 – 10.0 μM.

56. The composition of Claim 50, wherein said carnitine is provided at a concentration of about 1.0 – 100.0 μM.

57. The composition of Claim 50, wherein said serine is provided at a concentration of about 1.0 – 100.0 μM.

58. The composition of Claim 50, wherein said oleic acid is provided at a concentration of about 1.0 – 100.0 μM.

59. The composition of Claim 50, wherein said linoleic acid is provided at a concentration of about 1.0 – 100.0 μM.

60. The composition of Claim 50, wherein said arachidonic acid is provided at a concentration of about 1.0 – 100.0 μM.

61. The composition of Claim 50, wherein said α-tocopherol is provided at a concentration of about 0.1 – 10.0 μM.

62. The composition of Claim 50, wherein said ascorbic acid is provided at a concentration of about 0.005 – 5.0 mg/ml.

63. The composition of Claim 50, wherein said epidermal growth factor is provided at a concentration of about 0.1 – 10.0 ng/ml.